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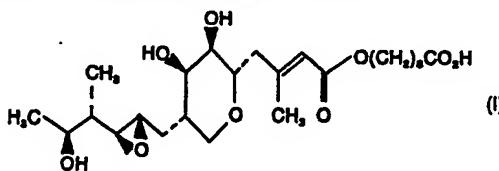
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㉓ Silver pseudomonate, compositions containing it and its use in treating pseudomonal infections.

㉔ Pseudomonic acid (I) is an antibiotic produced by aerobically culturing *Pseudomonas fluorescens*.



A process is provided for producing silver pseudomonate which process comprises reacting silver ions and pseudomonic acid or pseudomonate ions in aqueous solution and thereafter recovering the silver pseudomonate so formed.

Also provided is a method for treating wounds or burns infected with *Pseudomonas* organisms comprising administering a non-toxic anti-pseudomonally effective amount of silver pseudomonate to the wound or burn.

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TITLE MODIFI
see front pag

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COMPOUND AND USE

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08 The present invention relates to silver
09 pseudomonate, compositions containing it and its use in
10 treating pseudomonal infections.

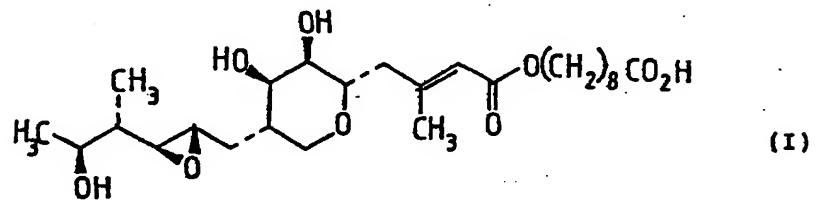
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12 Pseudomonic acid is an antibiotic produced by
13 aerobically culturing Pseudomonas fluorescens. The
14 compound, of formula (I) below, and its salts and
15 esters are disclosed and claimed in UK Patent No. 1 395
16 907.

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Whilst pseudomonic acid and its salts and esters
are active against a variety of human and animal
pathogens (see for instance UK Patent Nos. 1 577 730
and 1 577 545), they are not active at useful levels
against Pseudomonas species.

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Pseudomonas organisms tend to infect burns and
wounds. Such infections are often difficult to treat
as the organisms are not particularly susceptible to
antibiotics.

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02 It has now surprisingly been found that silver
03 pseudomonate is active against Pseudomonas organisms,
04 especially Pseudomonas aeruginosa, the causative agent
05 of 'blue pus' infections.

06

07 The silver salt of pseudomonic acid has not been
08 specifically disclosed in the above patents or any
09 other publications and is, therefore, novel.

10

11 Accordingly the present invention provides, in one
12 aspect, silver pseudomonate.

13

14 The invention also provides silver pseudomonate
15 for use in the treatment of the human or animal body.

16

17 Apart from its surprising activity against
18 Pseudomonas, silver pseudomonate has a similar spectrum
19 of activity against pathogens to those of pseudomonic
20 acid and sodium pseudomonate.

21

22 Accordingly the present invention also provides
23 silver pseudomonate for use in treating the human or
24 animal body, especially for treating infected wounds
25 and burns.

26

27 The invention also provides a process for
28 producing silver pseudomonate which process comprises
29 reacting silver ions and pseudomonic acid or
30 pseudomonate ions in aqueous solution and thereafter
31 recovering the silver pseudomonate so formed.

32

33 Suitably the process is effected by adding a
34 source of silver ions to an aqueous solution of
35 pseudomonic acid or a pseudomonate salt, especially
36 sodium pseudomonate.

37

Suitably the solution of pseudomonic acid or pseudomonate ions is the product of aerobically culturing Pseudomonas fluorescens (NCIB 10586). Such a solution may be the culture medium in which the organisms have been grown or it may have been produced by purifying such a medium for instance by extracting pseudomonic acid from such a culture medium using a polar, organic, water-immiscible solvent as described in EP 0 005 614. Alternatively the solution of pseudomonic acid or pseudomonate ions may be produced by dissolving pseudomonic acid or preferably a salt thereof, in an aqueous solvent. Preferably the solution is produced by dissolving pure sodium pseudomonate in water.

17 The source of silver ions is preferably a soluble
18 silver salt such as silver nitrate or silver carbonate.

20 The invention further provides silver pseudomonate
21 in substantially pure form, preferably at least 75%
22 pure, more preferably at least 90% pure, most
23 preferably at least 95% pure.

If precipitated from solution containing solvents other than water, the silver pseudomonate may be produced in a solvated form including a hydrated form. If precipitated from aqueous solution the silver pseudomonate may be in a hydrated form.

31 Accordingly the invention further provides,
32 solvated, including hydrated, silver pseudomonate.

34 Silver pseudomonate may be administered as the
35 pure compound (hereinafter referred to as the 'drug')
36 or it may be administered as a pharmaceutical
37 composition in association with a suitable carrier.

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02 Accordingly the invention also provides a
03 pharmaceutical formulation comprising silver
04 pseudomonate and a pharmaceutically acceptable carrier
05 therefor.

06

07 As used herein the term 'pharmaceutically
08 acceptable' includes 'veterinarily acceptable'.

09

10 The formulations may be adapted for administration
11 by any route, and would depend on the disease being
12 treated. Normally, the formulations will be presented
13 as topical solutions or suspensions for application to
14 the skin, ears or eyes. Alternatively the formulations
15 may be dry powders for application as an aerosol, or
16 they may be presented as impregnated dressings for
17 wounds and burns.

18

19 For topical application to the skin the drug may
20 be made up into a cream, lotion or ointment. Cream or
21 ointment formulations that may be used for the drug are
22 conventional formulations well known in the art, for
23 example, as described in standard text books of
24 pharmaceutics and cosmetics, such as Harry's
25 Cosmeticolgy published by Leonard Hill Books, and the
26 British Pharmacopoeia. Alternatively the drug may be
27 applied as a dry powder from an aerosol using
28 conventional diluents and propellants.

29

30 For topical application to the ear, the drug may
31 be made up into a solution or suspension in a suitable
32 liquid carrier, such as water, glycerol, diluted
33 ethanol, propylene glycol, polyethylene glycol or fixed
34 oils.

35

36 For topical application to the eye, the drug is
37 formulated as a solution or suspension in a suitable,
38 sterile aqueous or non-aqueous vehicle. Additives, for

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02 instance buffers such as sodium metabisulphite or
03 disodium edetate; preservatives including bactericidal
04 and fungicidal agents, such as phenylmercuric acetate
05 or nitrate or chlorhexidine, and thickening agents such
06 as hypromellose may also be included.
07

08 Particularly suitable topical formulations
09 comprise silver pseudomonate and at least 1% by weight
10 of a poly (substituted or unsubstituted alkylene)
11 glycol or a derivative thereof.
12

13 As used herein the term 'poly (substituted or
14 unsubstituted alkylene) glycol' refers to polymers
15 having the following repeating unit
16

17 - $(CH_2)_nO$ -
18

19 wherein n is an integer, preferably 2 or 3 and to such
20 polymers wherein one or more methylene groups of each
21 repeating unit is substituted. Suitable substituents
22 include alkoxy groups such as methoxy as in
23 polymethoxypropylene glycol. Such polymers are known
24 by a variety of names, for instance when n = 2, as
25 polyethylene glycol, polyoxyethylene, polyoxyethylene
26 glycol and macrogol and, when n = 3, as polypropylene
27 glycol, polyoxypropylene and polyoxypropylene glycol.
28 All these are useful in the invention as are
29 derivatives of these polymers.
30

31 Suitable derivatives include ethers and esters of
32 the poly (substituted or unsubstituted alkylene)
33 glycols, such as the macrogol ethers and esters, for

02 instance cetomacrogol, glycofurol, the 'Tweens'* and
03 block copolymers including poly (substituted or
04 unsubstituted alkylene) glycols such as Poloxamers
05 which are block copolymers of polyethylene glycol and
06 polypropylene glycol for instance the 'Pluronics'*, and
07 cross-linked polyethylene glycol.
08

09 The poly (substituted or unsubstituted alkylene)
10 glycols and derivatives thereof may be used singly or
11 various grades and types may be used in combination to
12 achieve the desired physical properties of the
13 formulation.
14

15 Preferably the formulation comprises polyethylene
16 glycol or a derivative thereof.
17

18 Suitably the formulation comprises from 0.01 to
19 50% by weight of silver pseudomonate, preferably 0.1 to
20 25%, more preferably 0.5 to 10% and most preferably
21 about 2% by weight of silver pseudomonate calculated as
22 the free acid. Such formulations comprising only
23 silver pseudomonate and a poly (substituted or
24 unsubstituted alkylene) glycol or derivative thereof
25 will, of course, contain up to 99.99% of the poly
26 (substituted or unsubstituted alkylene) glycol or
27 derivative thereof.
28

29 The formulation may comprise additional
30 therapeutic agents such as antibacterial, antifungal,
31 antiviral and antiinflammatory agents, for instance
32 chlortetracycline, miconazole, idoxuridine and
33 phenazone, provided that these are compatible with the
34

35 * 'Tween' and 'Pluronic' are trade names for the above
36 types of polymer.
37

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02 silver pseudomonate. Silver Pseudomonate tends to
 03 undergo a rearrangement reaction in the presence of
 04 acid and accordingly acidic agents are unlikely to be
 05 compatible with silver pseudomonate.

06

07 In a particular aspect the invention provides a
 08 topical formulation as described above wherein silver
 09 pseudomonate is the sole therapeutic agent.

10

11 In another aspect the invention provides a topical
 12 formulation comprising silver pseudomonate and at least
 13 1% by weight of polyethylene glycol or a derivative
 14 thereof.

15

16 Polyethylene glycols (PEG's) and derivatives
 17 thereof are commercially available in a variety of
 18 chain lengths and with a variety of consistencies, for
 19 instance:-

20

21 Polyethylene Glycols:-

22

23

Liquids	Semisolids	Hard Solids.
PEG 200	PEG 1000	PEG 4000*
PEG 300	PEG 1540	PEG 6000
PEG 400		

30

31

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Derivative	Chemical Composition	Consistency
Glycofurool	Tetrahydrofurfuryl alcohol polyethylene glycol ether	Liquid
Tween 60	Polyoxyethylene Sorbitan monostearate	Semi-solid
Tween 80	Polyoxyethylene Sorbitan monooleate	Liquid

45

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48

* PEG 4000 is the B.P. nomenclature for PEG with mean molecular weight of 3350. This material is also known as PEG 3350 in U.S.P. nomenclature.

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02 These may be used singly or admixed in suitable
03 proportions to achieve the desired consistency of
04 formulation.

05

06 The formulations of the present invention may
07 contain appropriate conventional additives such as
08 preservatives, solvents to assist drug penetration and
09 emollients in ointments and creams. The formulations
10 may also contain compatible conventional carriers, such
11 as cream or ointment bases and ethanol or oleyl alcohol
12 for lotions. Such carriers may be present as from
13 about 1% up to about 98% of the formulation. More
14 usually they will form up to about 80% of the
15 formulation.

16

17 Particularly suitable formulations according to
18 the present invention comprise at least 1% by weight of
19 PEG or a mixture of PEG's, from 0 to 25% by weight of a
20 PEG derivative or mixture of PEG derivatives and from
21 0.5 to 10% by weight of silver pseudomonate calculated
22 as the free acid.

23

24 Preferably the silver pseudomonate represents 1 to
25 5% of the formulation, most preferably about 2% of the
26 formulation calculated as the free acid.

27

28 Formulations of the invention may be produced by
29 conventional pharmaceutical techniques. Thus ointments
30 and creams are conveniently prepared by melting and
31 mixing together the solid or semi-solid PEG's or PEG
32 analogues or derivatives, and stirring in the
33 therapeutic agent and any other ingredients. The
34 product is then slowly cooled and filled into
35 containers such as collapsible metal or plastic tubes.

36

02 Liquid preparations, such as ear and eye drops,
03 are produced by dissolving the therapeutic agent in the
04 liquid PEG's or PEG analogues or derivatives and the
05 other ingredients are then added. The resulting
06 solution or suspension is distributed into glass or
07 plastic bottles or in single dose packs such as soft
08 gelatin capsules which are then heat sealed.
09

10 If necessary the formulation may be milled at any
11 suitable stage of the process.
12

13 A suitable sterilisation procedure may be included
14 in the above processes if necessary. Alternatively raw
15 materials are obtained in sterile conditions and the
16 formulations are produced aseptically.
17

18 The dosage employed for formulations administered
19 topically will, of course, depend on the size of the
20 area being treated. For the ears and eyes each dose
21 will typically be in the range from 10 to 100 mg of the
22 drug.
23

24 The present invention further provides a process
25 for producing a pharmaceutical formulation which
26 process comprises bringing into association silver
27 pseudomonate and a pharmaceutically acceptable carrier
28 therefor.
29

30 The present invention also provides a method for
31 treating pseudomonal infections of human or non-human
32 animals comprising administering a non-toxic
33 anti-pseudomonally effective amount of silver
34 pseudomonate to an infected human or non-human animal.
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02 In a particular aspect the invention provides a
03 method for treating wounds or burns infected with
04 Pseudomonas organisms comprising administering a
05 non-toxic anti-pseudomonally effective amount of silver
06 pseudomonate to the wound or burn.

07

08 Preferably the above methods are effected by
09 applying a topical formulation to the infected area.

10

11 The invention will now be illustrated with
12 reference to the following Examples and Biological
13 data.

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Example 1

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Silver Pseudomonate A

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07 Sodium pseudomonate A (1.82g, 4 mmol) and silver
08 nitrate (0.68g, 4 mmol) were stirred in distilled water
09 for 30 min resulting in the formulation of a white
10 gelatinous precipitate. The mixture was centrifuged,
11 the aqueous layer removed and the residue washed with
12 distilled water. The suspension was centrifuged and
13 the residual solid was dried over phosphorus pentoxide
14 under high vacuum for 2 days to yield silver
15 pseudomonate A, m.p. 164-166°C, (855 mg, 35%);
16 ν_{max} (KBr) 3400, 1710, 1645, 1515 cm^{-1} , $\delta_{\text{H}}(\text{CD}_3)_2\text{SO}$
17 5.68 (1H, s, H2), 2.12 (3H, s, CH_3 -15), 1.1 (3H, d,
18 CH_3 -14), 0.85 (3H, d, CH_3 -17) (Found: C, 49.6; H, 6.7;
19 Ag, 17.8.
20 $\text{C}_{26}\text{H}_{43}\text{O}_9\text{Ag}$ requires C, 51.4; H, 7.1; Ag, 17.8%).
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Example 2

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Liquid Formulation

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Silver pseudomonate may be dissolved in PEG 400 and the formulation adjusted, by addition of further PEG 400, to contain 2% by weight of silver pseudomonate.

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Example 3

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Ointment Formulation

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% w/w

15

PEG 400 59

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PEG 4000 39

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Silver pseudomonate 2

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The formulation may be produced by melting the mixture of PEG's and stirring in the silver pseudomonate.

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Example 4

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Lotion Formulation

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% w/w

27

PEG 400 74

28

Ethanol 24

29

Silver pseudomonate 2

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Example 5

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Drop Formulation

		% w/w
05		
06	PEG 400	74
07	Glycofurool	24
08	Silver pseudomonate	2

09

10 Example 6

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	% w/w
12	
13	Cetomacrogol emulsifying ointment
14	65
15	Polyethylene glycol 200
16	33
	Silver pseudomonate
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BIOLOGIGAL DATA

- a) The minimum inhibitory concentrations (MICs) of silver pseudomonate and sodium pseudomonate were determined against 20 strains of Pseudomonas aeruginosa in Blood Agar Base. Typical results are presented in Table 1. Silver pseudomonate was more active than sodium pseudomonate against all strains tested.
- b) MIC's of silver and sodium pseudomonate against various pathogenic bacteria were determined by standard methods. Typical results are presented in Table 2.

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Table 1

The activity of Sodium Pseudomonate and Silver
Pseudomonate against 20 strains of
Pseudomonas aeruginosa:

Typical MIC's

Pseudomonas aeruginosa	MIC* ug/ml	
	Sodium Salt	Silver Salt
NCTC 10662	12,800	128
Dalgleish	>128	128
PU7	>128	128
W985	>128	128
S41	>128	128
R60	>128	128
Pu4	>128	128
R59	>128	64
T3	>128	128
R3	6,400	128
R139	>128	128
R22	>128	128
W995	>128	128
59	>128	128
125	>128	128
4	>128	128
Fr13	6,400	128
D25	>128	128
ATCC 27853	>128	128
W996	>128	128

* MIC determined in serial dilution in Blood Agar Base. Inoculum of 0.001 ml of an overnight Tryptone Soya Broth Culture. Incubated at 37°C overnight.

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Table 2Typical MIC's (µg/ml) against Human Bacteria

Organism	Pseudomonate Salt, MIC (µg/ml)	
	Silver	Sodium
<i>E. coli</i> NCTC 10418	128	125
<i>P. mirabilis</i> 889	128	125
<i>K. aerogenes</i> A	128	250
<i>Ps. aeruginosa</i> NCTC 10662	128	12800
<i>Pasteurella multocida</i> 1633	0.5	0.25
<i>Haemophilus influenzae</i> Wy21	0.12	0.12
<i>Bacillus subtilis</i> 6633	0.25	0.25
<i>Corynebacterium xerosis</i> 9755	128	>125
<i>Staph. aureus</i> Oxford	0.5	0.25
<i>Staph. aureus</i> Russell	0.5	0.25
<i>Staph. aureus</i> W2827	0.5	0.25
<i>Strep. faecalis</i> I	64	50
<i>Strep. pyogenes</i> R80/421-A	0.25	0.25
<i>Strep. agalactiae</i> 2788-B	1.0	0.5
<i>Strep. spp.</i> 64/848-C	1.0	0.5

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Claims

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1. A compound silver pseudomonate.

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2. A compound as claimed in claim 1, being at least 75% pure.

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3. A compound as claimed in claim 1 or claim 2, being at least 90% pure.

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4. A compound as claimed in any one of claims 1 to 3, being at least 95% pure.

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5. A compound as claimed in any one of claims 1 to 4, being in the solvated form.

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6. A compound as claimed in any one of claims 1 to 5, being in the hydrated form.

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7. A process for producing silver pseudomonate which process comprises reacting silver ions and pseudomonic acid or pseudomonate ions in aqueous solution and thereafter recovering the silver pseudomonate so formed.

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8. A process as claimed in claim 7, effected by adding a source of silver ions to an aqueous solution of pseudomonic acid or a pseudomonate salt.

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9. A process as claimed in claim 7 or claim 8, wherein the pseudomonate salt is sodium pseudomonate.

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02

- 03 10. A process as claimed in claim 7 or claim 8,
04 wherein the solution of pseudomonic acid or
05 pseudomonate ions is the product of aerobically
06 culturing Pseudomonas fluorescens (NCIB 10586).
- 07 11. A process as claimed in any one of claims 7 to 10,
08 wherein the source of silver ions is a soluble
09 silver salt.
- 10 12. A process as claimed in any one of claims 7 to 11,
11 wherein the source of silver ions is silver
12 nitrate.
- 13 14. A pharmaceutical formulation comprising silver
15 pseudomonate and a pharmaceutically acceptable
16 carrier therefor.
- 17 18 19 14. A pharmaceutical formulation as claimed in claim
20 13, formulated for topical application.
- 21 22 23 15. A pharmaceutical formulation as claimed in claim
24 13 or claim 14, comprising silver pseudomonate and
25 at least 1% by weight of a poly (substituted or
26 unsubstituted alkylene) glycol or a derivative
27 thereof.
- 28 16. A pharmaceutical formulation as claimed in any one
29 of claims 13 to 15, comprising polyethylene glycol
30 or a derivative thereof.
- 31 32 17. A pharmaceutical formulation as claimed in any one
33 of claims 13 to 16, comprising silver pseudomonate
34 and at least 1% by weight of polyethylene glycol
35 or a derivative thereof.
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18. A pharmaceutical formulation as claimed in any one
03 of claims 13 to 17, wherein silver pseudomonate is
04 the sole therapeutic agent.

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19. A compound as claimed in claim 1, for use in the
07 treatment of the human or animal body infected with
08 Pseudomonas organisms.

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20. A compound as claimed in claim 19, for use in
treating infected wounds and burns infected with
11 Pseudomonas organisms.

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European Patent
Office

EUR PEAN SEARCH REPORT

Application number

EP 84 10 5070

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. *)
A	EP-A-0 005 614 (BEECHAM GROUP) * Claims 1,2 *	1,7	C 07 D 407/06 A 61 K 31/35
A	--- EP-A-0 068 680 (BEECHAM GROUP)		
D, A	--- GB-A-1 395 907 (BEECHAM GROUP) -----		
TECHNICAL FIELDS SEARCHED (Int. Cl. *)			
C 07 D 407/06			
<p>The present search report has been drawn up for all claims</p>			
Place of search BERLIN	Date of completion of the search 30-07-1984	Examiner PHILLIPS N.G.A.	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			